Amendments to the Specification:

Please amend the paragraph beginning at page 7, line 5 as follows:

~~Fig. 1 is~~ Figs. 1A-1H are a Hopp/Woods hydrophilicity profile of the amino acid sequence shown in SEQ ID NO:2. The profile is based on a sliding six-residue window. Buried G, S, and T residues and exposed H, Y, and W residues were ignored. These residues are indicated in the figure by lower case letters.

Please amend the paragraph beginning at page 11, line 30 as follows:

A representative human zvegf4 polypeptide sequence is shown in SEQ ID NO:2, and a representative mouse zvegf4 polypeptide sequence is shown in SEQ ID NO:53. DNAs encoding these polypeptides are shown in SEQ ID NOS:1 and 52, respectively. Analysis of the amino acid sequence shown in SEQ ID NO:2 indicates that residues 1 to 18 form a secretory peptide. The CUB domain extends from residue 52 to residue 179. A propeptide-like sequence extends from residue 180 to either residue 245, residue 249 or residue 257, and includes four potential cleavage sites at its carboxyl terminus, monobasic sites at residue 245 and residue 249, a dibasic site at residues 254-255, and a target site for furin or a furin-like protease at residues 254-257. Protein produced in a baculovirus expression system showed cleavage between residues 250 and ~~259~~ 249, as well as longer species with amino termini at residues 19 and 35. The growth factor domain extends from residue 258 to residue 370, and may include additional residues at the N-terminus (for instance, this domain may include residues 250 to 370 or residues 246 to 370). Those skilled in the art will recognize that domain boundaries are somewhat imprecise and can be expected to vary by up to ± 5 residues from the specified positions. Cleavage of full-length zvegf4 with plasmin resulted in activation of the zvegf4 polypeptide. By Western analysis, a band migrating at approximately the same size as the growth factor domain was observed. A matched, uncleaved full-length zvegf4 sample demonstrated no activation.

Please amend the paragraph beginning at page 15, line 17 as follows:

As noted above, residues 254-257 of SEQ ID NO:2 are believed to provide cleavage sites for furin or other proteases. However, polypeptides comprising a C-terminal interdomain region (e.g., $zvegf4_{52-257}$) can be prepared with or without one or more of residues 254-257 at the ~~amino~~ carboxyl terminus. In addition, polypeptides comprising another C-terminal interdomain region (e.g., $zvegf4_{52-245}$) can be prepared.

Please amend the paragraph beginning at page 21, line 33 as follows:

Amino acid sequence changes are made in zvegf4 polypeptides so as to minimize disruption of higher order structure essential to biological activity. As noted above, conservative amino acid changes are generally less likely to negate activity than are non-conservative changes. Changes in amino acid residues will be made so as not to disrupt the cystine knot and "bow tie" arrangement of loops in the growth factor domain that is characteristic of the protein family.

Conserved motifs will also be maintained. The effects of amino acid sequence changes can be predicted by computer modeling as disclosed above or determined by analysis of crystal structure (see, e.g., Lapthorn et al., *ibid.*). A hydrophilicity profile of SEQ ID NO:2 is shown in ~~Fig. 1~~ Figs. 1A-1H. Those skilled in the art will recognize that this hydrophilicity will be taken into account when designing alterations in the amino acid sequence of a zvegf4 polypeptide, so as not to disrupt the overall profile. Additional guidance in selecting amino acid subsitutions is provided by a comparison of the mouse (SEQ ID NO:53) and human (SEQ ID NO:2) zvegf4 sequences. The amino acid sequence is highly conserved between mouse and human zvegf4s, with an overall amino acid sequence identity of 85.1%.

Please amend the paragraph beginning at page 80, line 23 as follows:

A zvegf4 DNA fragment was generated by PCR (~~Advantage2~~ ADVANTAGE2 PCR Kit, Clontech, Palo Alto, CA) with 5' FseI and 3' AscI sites for direct cloning into the expression vector. The 5' primer contained an FseI site, Kozak sequence, and the first 21 basepairs of the native leader sequence for zvegf4 (ZC26,136; SEQ ID NO:43). The 3' primer contained the last 21 basepairs of zvegf4, a stop codon, and an AscI site (ZC26,137; SEQ ID NO:44). The PCR reaction included 1 ~~L~~ µL of template (ESTEP plasmid zvegf4pcrfl#3) and was run as follows: 94°C, 1 minute, 1 cycle; then 25 cycles of 94°C, 30 seconds; 55°C, 30 seconds; 68°C, 1 minute; then a final extension cycle of 72°C for 7 minutes.

Please amend the paragraph beginning at page 80, line 32 as follows:

The ESTEP plasmid zvegf4pcrfl#3 contains the full-length human zvegf4 fragment. This fragment was generated by PCR using 20 pm each of ZC22,341 (SEQ ID NO:45) and ZC22,342 (SEQ ID NO:46) primers and 3 ~~L~~ µL of a thyroid library. The reaction was run as follows: 94°C, 1 minute, 1 cycle; then 30 cycles of 94°C, 20 seconds; 66°C, 1.5 minutes; then a final extension cycle of 72°C for 5 minutes. The 1,272 bp product was gel purified on a 1% TBE gel, and the DNA was extracted from the gel slab using the ~~QIAquick~~ QIAQUICK Gel Extraction Kit (Qiagen, Valencia, CA). This 1,272 bp fragment was subcloned into pCR2.1 vector (Invitrogen, Carlsbad, CA), and designated zvegf4pcrfl#3.

Please amend the paragraph beginning at page 81, line 4 as follows:

The PCR generated fragment was purified (~~Qiaquick~~ QIAQUICK PCR clean-up kit, Qiagen, Valencia, CA) and digested with restriction enzymes AscI and FseI (New England Biolabs, Beverly, MA) in a single 100 ~~L~~ µL reaction. Five micrograms of the expression vector pEZE2 were also digested with FseI and AscI in a single 100 ~~L~~ µL reaction. The digested DNA was fractionated by agarose gel electrophoresis and the DNA fragments were isolated and purified (~~Qiaquick~~ QIAQUICK Gel Extraction Kit, Qiagen).

Please amend the paragraph beginning at page 81, line 10 as follows:

Five microliters of the zvegf4 DNA fragment and 1 ~~:L~~ µL of the pEZE2 vector fragment were ligated overnight at room temperature (New England Biolabs High Concentrated Ligase and supplied buffer). One microliter of the ligation reaction was added to 25 ~~:L~~ µL of electrocompetant *E. coli* strain DH10B (Life Technologies) in a 0.2 cm cuvette. The mixture was electroporated (BioRad *E. coli* Pulser) at 2.3 kv. To the cuvette, 1 mL of LB broth was added, and 100 ~~:L~~ µL of the mix was plated onto LB/Ampicillin agar plates. The plates were incubated overnight at 37°C, and 8 isolated colonies were picked for DNA mini prep (~~Qiaquick~~ QIAQUICK Mini-Prep Kit, Qiagen). Individual clones were screened by PCR for the presence of zvegf4 DNA, using the above-mentioned primers. DNA sequencing was performed on clones #1-6, to verify the correct full-length sequence. One clone contained the correct expected sequence and a Maxi prep of DNA was made (Qiagen Plasmid Maxi Kit, Qiagen).

Please amend the paragraph beginning at page 81, line 22 as follows:

CHO DG44 (Chasin et al., *Som. Cell. Molec. Genet.* 12:555-666, 1986) were plated and allowed to grow to approximately 50% to 70% confluency over night at 37°C in MEM alpha media (JRH Biosciences, Lenexa, KS), 7.5% fetal bovine serum (Hyclone, Logan, UT), 1% L-glutamine (Life Technologies), 1% sodium pyruvate (Life Technologies), 1% HT solution (Life Technologies), and 1% Penicillin/Streptomycin (Life Technologies). The cells were then transfected with the plasmid pEZE2/zvegf4 by liposome-mediated transfection, using a 10:1 (w/w) liposome formulation of the polycationic lipid dioctaldecylamidoglycyl spermine, in serum-free (SF) medium formulation DMEM/F12 – Life Technologies, Non-Essential Amino Acids-Life Technologies, 1% L-glutamine, 1% sodium pyruvate. The plasmid pEZE2/zvegf4 was diluted in a final volume of 500 :L of SF medium in a 15 mL conical tube, and 20 ~~:L~~ µL of ~~Transfectam~~ TRANSFECTAM (Promega, Madison, WI) reagent was added, mixed well and incubated at room temperature for 10 minutes. After incubation, 4.5 mL of SF medium was added to the DNA mixture and mixed well using a 5 mL pipette. The cells were rinsed 3 times with SF medium, and the 5 mL of DNA solution was overlayed upon the cell monolayer. The cells were incubated at 37°C, 5% CO_2 for 2 hours. Then 6 mL of complete medium (MEM alpha, 7.5% FBS, 1% L-glutamine, 1% sodium pyruvate, 1% HT, 1% Pen/Strep) and the cells were incubated for a further 48 hours. After 48 hours, the cells were trypsinized from the plate with 1 mL of 0.25% Trypsin/ 1 mM EDTA (Life Technologies) and quenched with 4 mL of complete medium without nucleosides (MEM alpha, 7.5% Dialysed FBS, 1% L-glutamine, 1% sodium pyruvate, 1% Pen/Strep). Five hundred microliters of the cell suspension were transferred to plates containing 10 mL of complete medium without nucleosides. The cultures were grown for 14 days, until single colonies that were approximately 0.25 cm in diameter were present. Cloning rings (Bellco Glass, Inc., Vineland, NJ) were used to isolate 24 single colonies, which were removed with trypsin, transferred to 6 well cell cluster plates (Costar, Corning, NY), and incubated 4 days.

Please amend the paragraph beginning at page 82, line 12 as follows:

The cell wells were rinsed with SF medium and 2 mL of SF medium was added, and the culture was incubated for 24 hours. The conditioned SF medium was concentrated approximately 20-fold using a 10K centrifuge device (Millipore Corporation, Bedford, MA). Twenty-five microliters of the concentrate was added to 15 ~~L~~ mL of 4X Sample Buffer (Novex, San Diego, CA) with 50 mM beta-Mercaptoethanol, and the mixture was run on a 4-12% ~~NuPAGE~~ NUPAGE gel (Novex). The proteins from the gel were transferred to nitrocellulose membranes (Novex) and the blot was blocked with 10% non-fat dry milk in Western A (0.25% gelatin, 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.05% Igepal CA-630) overnight at room temperature on a rotating shaker platform. The membrane was rinsed 3 times in Western A. An antibody to the N-terminus of the zvegf4 protein was diluted at 1:3000 in 50 mL 5% non-fat milk in Western A. The antibody solution was overlayed on the membrane and incubated at room temperature on a rocking platform for 1 hour. After the 1 hour incubation, the solution was discarded and the membrane rinsed 3 times with Western A and once with Western B (50 mM Tris pH 7.4, 5 mM EDTA, 0.05% Igepal CA-630, 1 M NaCl, 0.25 % Gelatin). The secondary antibody, an $F(ab')_2$ fragment of Donkey-Anti-Rabbit-HRP (Amersham Corp., Arlington Heights, IL), was diluted in Western A at 1:3000, overlayed on the membrane, and incubated 1 hour at room temperature on a rocking platform. The secondary antibody solution was discarded, and the membrane was washed 3 times in Western A and 3 times in Western B. Chemiluminescence was used to detect the full-length or protease-digested N-terminus of zvegf4 according to the manufacturer's instructions (Pierce, Rockford, IL), and was analysed by ~~LumiAnalyser~~ LUMIANALYSER (Roche/Boehringer Mannheim, Mannheim, Germany). Four of the 12 clones were positive for zvegf4, and numbers 7 and 12 were trypsinized and transferred to T175 flasks (Costar, Corning, NY) in complete medium without nucleosides.

Please amend the paragraph beginning at page 83, line 25 as follows:

A partial mouse zvegf4 sequence was obtained by probing a mouse genomic library with a human zvegf4 restriction digest fragment containing the entire coding sequence. The probe was generated by digesting 8 ~~g~~ µg of a full-length human zvegf4 plasmid with EcoR1 (Gibco BRL, Gaithersburg, MD). The 1,289 bp fragment was gel purified on a 2.3% TBE gel and the cDNA was extracted from the agarose slab using the ~~QIAquick~~ QIAQUICK Gel Extraction Kit (Qiagen). The mouse genomic library was an embl3 SP6/T7 lambda BamH1 cloned library (Clontech, Palo Alto, CA) plated on a K802 host lawn on 24 NZY plates, and represented 7.2 x 10^5 pfus.

Please amend the paragraph beginning at page 83, line 33 as follows:

~~Twenty four~~ Twenty-four filter lifts were prehybridized in EXPRESSHYB solution (Clontech) containing 0.1 mg/ml salmon sperm DNA which had been boiled 5 minutes, then iced. Hybridization took place overnight at 50°C. Sixty three ng of the human fragment mentioned

above were labeled with ^{32}P using the ~~Rediprime~~ REDIPRIME II Random Prime Labeling System (Amersham Pharmacia, Buckinghamshire, England). Unincorporated radioactivity was removed using a NucTrap push column (Stratagene, La Jolla, CA). Filters were hybridized in EXPRESSHYB solution containing 1.0 x 10^6 cpm/ml zvegf4 probe, 0.1 mg/ml salmon sperm DNA, and 0.5 ~~:g/ml~~ µg/ml murine cot-1 DNA which had been boiled 5 minutes, then iced. Hybridization took place overnight at 50°C. Filter lifts were washed in 2 x SSC, 0.1% SDS at room temperature for 2 hours, then the temperature was raised to 60°C for one hour. Overnight exposure at -80°C showed 7 putative primary hits.

Please amend the paragraph beginning at page 84, line 8 as follows:

A K802 host culture was prepared to plate the primary hits for a secondary screen. The 7 primary hits were picked with a Pasteur pipet and eluted in 1 ml SM (0.1 M NaCl, 50 mM Tris pH 7.5, 10 mM ~~MgSO4~~ $MgSO_4$, 0.02% gelatin) with a few drops of chloroform overnight at 4°C. After plating to determine titers, 10 times the number of plaques in the original pfu were plated on NZY maxi plates with 10 mM ~~MgSO4~~ $MgSO_4$/NZY top agarose and a lawn of K802 cells for four of the primary hits and grown overnight at 37°C. Lifts were done using ~~Hybond-N~~ HYBOND-N filters (Amersham Pharmacia). The filters were marked for orientation with a hot needle, denatured in 1.5 M NaCl and 0.5 M NaOH for 10 minutes, then neutralized in 1.5 M NaCl and 0.5 M Tris-HCl pH 7.2 for 10 minutes. The DNA was affixed to the filter using a STRATALINKER UV crosslinker (Stratagene, La Jolla, CA) at 1200 joules, and prewashed at 65°C in prewash buffer consisting of 0.25 x SSC, 0.25% SDS and 1 mM EDTA, changing solution three times for a total of 45 minutes to remove cell debris. Five lifts were put in each vial, three vials total. Each vial of lifts was prehybridized overnight at 50°C in 13 ml of EXPRESSHYB Hybridization Solution (Clontech) mixed with 1.3 mg salmon sperm DNA which had been boiled 5 minutes, then iced.

Please amend the paragraph beginning at page 84, line 24 as follows:

~~Sixty-three~~ Sixty-three ng of the human zvegf4 fragment was labeled for a probe as described above. Each vial of filters was hybridized in 9 ml of EXPRESSHYB Hybridization Solution mixed with 0.99 to 1.1 x 10^6 human zvegf4 probe, 0.5 ~~:g/ml~~ mg/ml murine cot-1 DNA, and 0.9 mg/ml salmon sperm DNA which had been boiled 5 minutes, then iced. Hybridization took place overnight at 50°C. Wash conditions described above for the primary screen were repeated for this secondary screen. Two of the 4 primary putative hits that were tested came up positive after an overnight exposure at -80°C.

Please amend the paragraph beginning at page 84, line 32 as follows:

Isolated plaques #7c1 and #18b2 were eluted in 200 ~~:l~~ µl SM overnight at 4°C, and fresh host K802 cells were prepared. Serial dilutions ranging from 10^{-2} to 10^{-3} were plated to obtain a titer estimate. Only #18b2 gave any plaques (for a titer of 2.6 to 3.0 x 10^3 phage per ~~:l~~

µl), and this plaque was further pursued. Two plates with 10^5 pfus per plate were prepared for a phage DNA prep from plate lysates. Plates were grown at 37°C for 6 hours, until the phage were starting to get confluent, and then 12 ml of SM per plate was added to elute the phage overnight at 4°C. At this point, plates were shaken at room temperature one hour, the supernatant was removed, 1% chloroform was added, and supernatant was shaken for 15 minutes. The DNA was prepped using the ~~Wizard~~ WIZARD Lambda Preps DNA Purification System (Promega), sections IV and VI.

Please amend the paragraph beginning at page 85, line 6 as follows:

Plaque #18b2 DNA was cut with several restriction enzymes to generate fragments to run on a Southern gel. Digests were run on a 1% TBE agarose gel. The gel was soaked in 0.25 M HCl for 30 minutes, rinsed in distilled H_20, soaked in 0.5 M NaOH and 1.5 M NaCl for 40 minutes with one solution change, and neutralized in 1.5 M NaCl and 0.5 M Tris-HCl (pH 7.2) for 40 minutes with one solution change. A TURBOBLOTTER Rapid Downward Transfer System (Schleicher & Schuell, Keene, NH) was set up to transfer the DNA onto a Nytran/BA-S membrane (Schleicher & Schuell) overnight. The DNA was affixed to the Nytran using a STRATALINKER UV crosslinker (Stratagene) at 1200 joules. The blot was prehybridized overnight at 50°C in 12 ml EXPRESSHYB Hybridization Solution (Clontech) mixed with 1.2 mg salmon sperm DNA which had been boiled 5 minutes, then iced. Fifty nine ng of the human zvegf4 fragment was labeled for a probe, as described above. Unincorporated radioactivity was removed by chromatography using a commercially available push column (NUCTRAP column, Stratagene). Ten ml of EXPRESSHYB Hybridization Solution was mixed with 1.0 x 10^6 cpm/ml of human zvegf4 probe, 0.5 ~~÷g/ml~~ µg/ml murine cot-1 DNA, and 0.1 mg/ml salmon sperm DNA which had been boiled 5 minutes, then iced, and then added to the blot. Hybridization took place overnight at 50°C. The blot was washed as described above, and exposed to film overnight at -80°C.

Please amend the paragraph beginning at page 85, line 24 as follows:

The Southern gel had a fragment from the BamH1/Pst1 digest which hybridized to the probe in the size range of 2.0 to 2.9 kb, which was pursued. Plaque 18b2 lambda DNA (2.8 ~~÷g~~ µg) was cut with 20 units of BamH1 (Boehringer Mannheim, Indianapolis, IN), and 20 ~~÷l~~ µl Pst1 (Life Technologies) for 2 hours at 37°C. The digest was run on a 1% TBE gel, and a 2.0 kb doublet, as well as 2.7 kb/2.9 kb bands, were excised from the gel. The DNA was extracted from the agarose using the ~~Qiaquick~~ QIAQUICK Gel Extraction Kit (Qiagen). The 18b2 fragments were ligated into a pbluescriptIIKS+ vector (Stratagene) cut with BamH1, Pst1 and BamH1/Pst1. Three clones with a Pst1 insert, and 4 clones with a BamH1/Pst1 insert, from these ligations were digested with their respective insert site restriction enzymes for another Southern blot to determine which was the original hybridizing fragment. The 1% TBE gel was treated and the DNA was transferred to the Nytran blot as described above. The blot was prehybridized as above

in 13 ml of hybridization solution. Fifty nine ng of the human zvegf4 fragment was labeled and unincorporated radioactivity was removed as described above. Human zvegf4 probe (8.4 x 10^5/ml cpm), 0.1 mg/ml of salmon sperm DNA, and 0.5 ~~:g/ml~~ µg/ml of mouse cot-1 DNA were boiled 5 minutes, iced 1 minute, and mixed with 7 ml of EXPRESSHYB hybridization solution, then added to the blot. Hybridization took place overnight at 50°C. The same washing procedure was used as mentioned above. The blot was exposed to film for 3 hours at -80°C, and both 2.0 kb band inserts strongly hybridized to the probe. These clones were sequenced and found to contain part of the murine zvegf4 cub domain. Primers were designed from this sequence for a PCR cDNA screen.

Please amend the paragraph beginning at page 86, line 19 as follows:

The ~~in-house~~ in-house mouse testis arrayed library representing 9.6 x 10^5 clones was screened by PCR using primers ZG26,317 (SEQ ID NO:49) and ZG26,318 (SEQ ID NO:50) according to conditions specified above. This library was deconvoluted down to a positive pool of 250 clones. *E. coli* DH10B cells (Gibco BRL) were transformed with this pool by electroporation following the manufacturer's protocol. The transformed culture was titered and arrayed out to 96 wells at ~20 cells/well. The cells were grown up in LB+amp overnight at 37°C. An aliquot of the cells was pelleted and PCR was used to identify a positive pool. Thermocycler conditions were as described above. The remaining cells from a positive pool were plated, and colonies were screened by PCR to identify a positive clone. Sequence analysis indicated that this clone, named "zvegf4mpzp7x-6", was incomplete at the 5' end and appeared to contain an intron at the 5' end.

Please amend the paragraph beginning at page 86, line 31 as follows:

The mouse salivary gland library representing 9.6 x 10^5 clones was then screened by PCR using primers ZG26,317 (SEQ ID NO:49) and ZG26,318 (SEQ ID NO:50) according to conditions specified above. The library was deconvoluted down to a positive pool of 250 clones. This 250 clonal pool was verified as having the 5' end by RACE. Twenty pm each of ZG26,318 (SEQ ID NO:50) and ZG14,063 (SEQ ID NO:51) primers and 3 ~~:l~~ µl of that pool was used. The reaction was run as follows: 94°C, 2 minutes, then 5 cycles of 94°C, 15 seconds; 70°C, 30 seconds; 30 cycles of 94°C, 15 seconds, 62°C, 20 seconds; 7°C, 30 seconds, and a final extension at 72°C for 7 minutes. The RACE product obtained upon sequencing confirmed that this pool contained the initiation Met. The same protocol as described above was carried out to isolate a single clone from the pool. Sequence analysis revealed that this clone, named "zvegf4mpzp7x-7", had a 225 bp deletion in coding compared to clone #6 (bp 865 to bp 1079 in the final sequence).

Please amend the paragraph beginning at page 87, line 12 as follows:

The ~~full-length~~ full-length cDNA clone was generated by a two step ligation of fragments from clone #6 and clone #7 from above. An EcoR1/Hind3 three prime fragment was

generated from clone #6 first. Nine ~~g~~ µg of clone #6 were digested with 15 units of EcoR1 (Gibco BRL, Gaithersburg, MD) and 15 units of Hind3 (Gibco BRL) for 2 hours at 37°C. The 528 bp fragment was gel purified on a 1% TBE gel, and the cDNA was extracted from the gel slab using the ~~QIAquick~~ QIAQUICK Gel Extraction Kit (Qiagen). It was ligated into pbluescriptIIKS+ (Stratagene) digested with EcoR1 and Hind3. Three ~~g~~ µg of a clone with this zvegf4 insert was digested with 15 units of EcoR1 (Gibco BRL), gel purified on a 1% TBE gel, and the DNA was extracted using the kit mentioned above. The 5' EcoR1 zvegf4 fragment from clone #7 was ligated into the EcoR1-digested clone mentioned above. This EcoR1 fragment was generated by digesting 8 ~~g~~ µg of clone #7 with 30 units of EcoR1 (Gibco BRL) for 2 hours at 37°C. The 754 bp fragment was gel purified on a 1% TBE gel, and the DNA was extracted from the gel slab as mentioned above.